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PATENT

Attorney Reference Number 7946-79836-01
Application Number 10/696,909

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Lorens et al.
Application No. 10/696,909
Filed: October 29, 2003
Confirmation No. 9257
For: MODULATORS OF ANGIOGENESIS AND TUMORIGENESIS
Examiner: Peter J. Reddig
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DECLARATION OF DR. SACHA HOLLAND UNDER 37 C.F.R. § 1.132

1. I, Sacha Holland, Ph.D., am a joint inventor of the above-identified patent application.

2. I have read and understood the above-identified patent application, including the pending claims, and the Office action dated December 12, 2007.

3. It is my understanding that in the December 12, 2007 Office action, the pending claims were rejected, in part because of alleged lack of enablement due to the absence of data showing that compounds identified by the claimed methods inhibit angiogenesis *in vivo*.

4. *In vivo* experiments were carried out under my direction to test the efficacy of compounds as inhibitors of angiogenesis, utilizing compounds identified as inhibitors of Axl polypeptide in cell-based *in vitro* assays, such as inhibition of haptotaxis, cell proliferation, and endothelial tube formation in endothelial cells and Axl kinase activity in HeLa cells (such as those described in the application at page 30, line 5 to page 31, line 32).

5. The results of some of these *in vivo* experiments were included in our publication, Holland *et al.*, *Cancer Res.* 65:9294-9303. October 15, 2005, which is submitted herewith as **Exhibit A.**

6. Holland *et al.* describes use of a short hairpin RNA (shRNA) against Axl in a mouse sponge angiogenesis assay (paragraph bridging page 9295-9296). Briefly, human microvascular endothelial cells (HMVEC) infected with an Axl shRNA vector were seeded into sponge matrices with Matrigel and implanted subcutaneously in severe combined immunodeficient (SCID) mice. After 14 days, the sponges were harvested and markers of angiogenesis were analyzed. These data are described on page 9297-9298, under the heading "Axl knockdown impairs blood vessel formation and function in a mouse angiogenesis model." In implants containing the Axl shRNA, neovascularization was reduced, as shown by a decrease in expression of human Tie-2, which identifies human endothelial cells (Fig. 4C*i*). There was also a decrease in human UEA-1 lectin fluorescence in the Axl shRNA infected implants (Fig. 4C*ii*), indicating a reduction in functional circulation in the neovasculature. Finally, vessel morphology of the Axl shRNA infected implants, as assessed by anti-human CD31 staining, showed that the vessels generated were smaller than controls and lacked patent lumens (Fig. 4D).

7. Holland *et al.* also describes use of an Axl shRNA in a mouse tumor xenograft assay (page 9296, paragraph headed "Xenograft assay"). MDA-MB-231 breast carcinoma cells were infected with Axl shRNA and implanted in CB-17 SCID mice. Tumor growth was measured for 30 days, or until the tumor reached a volume of 2000 mm^3. These data are described on page 9298 under the heading "Inhibition of Axl expression reduces growth of MDA-MB-231 breast carcinoma cells in a xenograft assay." Cells infected with Axl shRNA showed an impaired ability to grow as xenografts in SCID mice (Fig. 5B), although their ability to grow in culture was not impaired (page 9298, second column, lines 1-3).

8. In additional experiments, small molecule inhibitors of *in vitro* Axl kinase activity in a cell-based assay were tested for *in vivo* inhibition of angiogenesis. Prepared Hydron pellets containing basic fibroblast growth factor (bFGF) were implanted into a corneal pocket cut in one eye of female C57BL/6 mice. Test compounds R562 or R572, a positive control agent (Sutent®), or vehicle (0.5% hydroxypropyl methyl cellulose (HPMC)/0.1% Tween® 80; acidified with tartaric acid for R562) was administered by oral gavage twice a day for 5 days beginning the day after surgery. On day 6 average vessel length from limbal vessels towards the

pellet and continuous circumferential zone (CH = clock hours) were measured and area of neovascularization was calculated using the formula: Area (mm^2) = πVL x CH. Both R562 and R572 (at 50 mg/kg and 100 mg/kg) significantly reduced corneal neovascularization in the corneal pocket assay.

9. In additional experiments on tumor growth *in vivo* utilizing small molecule inhibitors of Axl kinase activity, female nude mice (nu/nu, Harlan) were injected with 5 x 10^6 MDA-MB-231 breast carcinoma cells in the right flank. Alternatively, mice were implanted with 1mm^3 Caki-1 renal carcinoma tumor fragments. Growth was monitored until the average size approached 80-120mm^3, at which time mice were sorted by tumor size into treatment groups. R562, R572, a positive control agent (Sutent®), or vehicle (0.5% HPMC/0.1% Tween® 80; acidified with tartaric acid for R562) was administered twice a day by oral gavage. Tumors were measured twice weekly using calipers. Partial regression is defined as tumor volume $\leq$ 50% of day 1 volume for 3 consecutive measurements but $\leq$ 13.5 mm^3 for at least one of these measurements. Complete regression is defined as tumor volume < 13.5 mm^3 for 3 consecutive measurements. R562 (125 mg/kg) inhibited the growth of MDA-MB-231 breast carcinoma tumor xenografts and increased survival time of tumor xenograft animals. Further, one partial and one complete regression were noted. R572 (100 mg/kg) inhibited the growth of Caki-1 renal carcinoma xenografts.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Sacha Holland, Ph.D.

3.11.08
Date